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Microbicides to prevent heterosexual transmission of HIV: Ten years down the road

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Abstract

The development of topical microbicides for HIV prevention originated in response to the unabated spread of HIV despite the availability of an effective HIV prevention tool (condoms), as well as the lack of an effective HIV vaccine. Initially, hopes were pinned on existing over-the-counter spermicides containing nonoxynol-9. Concern about the toxicity of nonoxynol-9 with frequent use, and its small or nonexistent protective effect against HIV and other sexually transmitted infections (STIs), has spurred the development of new microbicides with a number of novel mechanisms of action. Significant progress has been made in the last decade. The microbicides pipeline currently contains approximately 34 products in preclinical development, 15 in phase I safety trials, four in phase II expanded safety and preliminary effectiveness trials, and three in phase II/III or phase III effectiveness trials. Laboratory and clinical research has been complemented by a growing body of research and literature on microbicide acceptability, harm reduction and dual protection strategies, and potential markets. However, many challenges remain, including the need for a significant increase in investment to accelerate product development and complementary research, and to plan for availability and access once effective microbicides are available.

Introduction

Over 36 million people worldwide are living with HIV/AIDS, and over 22 million people have died from AIDS since the beginning of the epidemic (*1*). The proportion of women living with HIV has risen steadily in recent years. Five years ago, 41% of HIV-positive adults were women; by 2000, that number had risen to 47% (*2*). In sub-Saharan Africa, where the primary mode of HIV transmission is heterosexual intercourse, 55% of HIV-positive adults are women (*1*).

The main HIV prevention tools--condoms, reducing the number of sexual partners, and treatment of reproductive tract infections--are not feasible for many women (*3*, *4*). After 2 decades of male condom promotion, the absolute number of male condoms used worldwide has increased dramatically. However, consistent condom use remains difficult to achieve, and resistance to condom use in some settings, such as primary partnerships, remains high. Women often have limited ability to get their male partners to use condoms due to social, cultural, and economic gender inequalities. In some countries, the female condom has increased options for protection against HIV somewhat, but problems with long-term acceptability have been reported, and female condoms cannot be used without the cooperation of men (*6*, *7*).

Reducing the number of sexual partners may not be feasible for those women who, because of limited educational and employment opportunities, are financially dependent on their male partners. Furthermore, a significant proportion of women are infected by their husbands. Even when women themselves are monogamous, their partners may not be.

Finally, women often are not treated for reproductive tract infections, or treatment is delayed. The majority of reproductive tract infections in women are asymptomatic, which makes women less likely to seek treatment and makes diagnosis difficult in the absence of laboratory testing (*8*). Although research on a preventive HIV vaccine is critically important and moving forward, it will be some time before a vaccine is available and accessible (*9*).

There is, therefore, an urgent need to extend the range of prevention methods available, particularly those that women can control. A microbicide is a product that is applied topically inside the vagina or rectum to prevent infection with HIV and potentially a number of bacterial and viral sexually transmitted infections (STIs). They may take any of a number of forms, including gels, creams, or suppositories, and may or may not be spermicidal. In the case of vaginal sex, microbicides are inserted by women and therefore only require passive acquiescence of men. Microbicides could be used alone, or in combination with a physical barrier, to provide increased protection or backup in case of barrier failure (*10*). For many women and couples, the importance of having children is a major obstacle to condom use, and noncontraceptive microbicides would give them an option with which to protect themselves from HIV while trying to conceive (*11*). By reducing the risk of HIV infection in women, microbicides would contribute to a reduction in mother-to-child transmission. They may also prevent transmission from women to their male partners and reinfection in women who are already HIV-positive. Additional research is investigating ways that microbicides can be formulated for use in the rectum during anal sex (*12*).

What is in the microbicides pipeline?

The identification of novel microbicidal compounds is one of the most rapidly expanding areas of HIV prevention research. In 1994, only a dozen compounds were in preclinical stages of development, with just eight ready for phase I safety trials and none in later stages of clinical testing. Today, according to the Alliance for Microbicide Development, 38 biotech companies, 28 not-for-profit groups, and seven public-sector agencies are investigating microbicides (*13*). An estimated total of 56 products are currently in the pipeline: 34 are in preclinical stages of development, 15 are in phase I safety trials, four are in phase II expanded safety and preliminary effectiveness trials (Savvy cream, Emmelle gel, *Lactobacillus crispatus* suppository, and Praneem Polyherbal suppository), and three are about to enter phase II/III (BufferGel and Pro-2000 gel) or phase III (Carraguard gel) effectiveness trials (*13*).

Microbicidal candidates fall into four categories--or a combination of categories--based on their mechanism of action: 1) products that kill or inactivate infectious pathogens; 2) products that block fusion, i.e., attachment of pathogens to the mucosal surface of target cells; 3) products that inhibit postfusion activity; and 4) products that enhance naturally occurring vaginal defense mechanisms (*13*, *14*). Approximately 35 of the products currently in the pipeline are contraceptive as well as microbicidal, and 21 are noncontraceptive (*13*).

1. Compounds and products that kill or inactivate infectious pathogens

Table 1 summarizes compounds that kill or inactivate HIV and other STI pathogens. This category includes compounds that disrupt lipid cell membranes (surfactants or detergents), increase membrane porosity (peroxidases/peroxides, antimicrobial peptides), cause agglutination (monoclonal antibodies), maintain an acidic pH in the vagina (acidic buffers), or coat cells (lipids). A few plant extracts have been identified that kill or inactivate pathogens, probably through one or more of these mechanisms of action.

Table 1. Compounds and products that kill or inactivate pathogens.

Product Category	Examples
Surfactants/detergents	Nonoxynol-9, octoxynol-9, benzalkonium chloride, menfegol, Savvy (C31G), sodium dodecyl sulfate (SDS), sodium laurel sulfate (SLS), chlorhexidene
Peroxidases/peroxides	Haloperoxidases, halides
Lipids	Hydrogels, synthetic lipids adapted from human breast milk lipids, hemicholinium and related lipids
Plant extracts	Praneem polyherbal suppository, gossypol
Antimicrobial peptides	(See compounds that enhance vaginal defense mechanisms)
Monoclonal antibodies	(See compounds that enhance vaginal defense mechanisms)
Acidic buffers	(See compounds that enhance vaginal defense mechanisms)

Early hopes were pinned on existing over-the-counter spermicides containing nonoxynol-9 as potential microbicides. Nonoxynol-9 is a surface-active agent that destroys cell membranes of pathogens as well as those of bystander genital mucosal cells. Recent studies of a number of over-the-counter nonoxynol-9 products have demonstrated that they are ineffective against HIV and most other STIs, and they also increase the risk of genital ulceration when used frequently (*15*-*18*). Similarly, a safety study of menfegol was terminated early due to increased genital ulceration with frequent use (*19*). Despite these setbacks, work continues on potentially less irritating surfactants, and new delivery systems that are designed to release the surfactant in lower concentrations over an extended period of time. Researchers at Biosyn (Huntingdon Valley, Pennsylvania, United States), for example, are conducting early clinical trials of Savvy. Savvy (C31G) is formulated as a cream with a mixture of two synthetic surface-active molecules, which have shown effectiveness in killing HIV and a variety of other pathogens (*20*).

The Talwar Research Foundation in India has developed a polyherbal suppository with anti-HIV activity, made from purified neem, reetha, and cinchona bark extracts. Early clinical trials are currently being conducted in India, Brazil, China, the Dominican Republic, Egypt, and Nigeria (*13*).

2. Inhibitors of pathogen attachment to target cells

The second group of compounds works by inhibiting attachment of the pathogen to target mucosal cells, also referred to as fusion (Table 2). Some agents in this class specifically prevent attachment of HIV to its target cells by either blocking HIV surface

proteins or HIV receptors on target cells. Others function nonspecifically by coating pathogens or target cells or both through charged interactions.

Table 2. Inhibitors of pathogen attachment to target cells.

Product Category	Examples
Fusion blockers specifically targeting HIV surface proteins or HIV receptors	gp-41 inhibitor (T-20), CCR-5 inhibitor
Other fusion blockers (often active against multiple organisms)	Cyanovirin-N (CV-N), beta-lactoglobulin (B69), B195/CAP (cellulose acetate phthalate)
Non-specific blockers (active against multiple organisms)	Sulfated/sulfonated polymers (Carraguard, Emmelle, Ushercell, Pro-2000), Q-2 bioadhesive polysaccharide, other charged polymers

Cyanovirin-N is a protein isolated from cultures of freshwater blue-green algae or produced by recombinant DNA techniques. The U.S. National Cancer Institute discovered that it binds irreversibly to the HIV gp120 surface receptor and therefore shows promise as a microbicide (*21*). The modified protein known as B69 was developed by researchers at the New York Blood Center through the chemical modification of bovine beta-lactoglobulin, a major protein of milk and whey. This protein has been shown to block the CD4 receptor on HIV target cells. An added advantage is that it can be produced inexpensively in large quantities (*22*). The New York Blood Center is also evaluating cellulose acetate phthalate (CAP or B195) for use as a microbicide. CAP is a common pharmaceutical excipient used for enteric film coating of capsules and tablets. Recent research has shown that it binds to gp120 and to a variety of HIV receptors on target cells (*23*). Extensive preclinical studies on B69 and CAP/B195 are in progress.

The nonspecific blockers include a few products that are the farthest along in the microbicides pipeline, including Carraguard, Emmelle, Pro-2000, and Ushercell. All four products are sulfated or sulfonated polymers. Carraguard gel is made of carrageenan derived from seaweed and is under development by the Population Council (New York, NY, USA). It blocks infection with HIV, herpes simplex virus type 2, human papillomavirus, and *Neisseria gonorrhoeae* in in vitro and animal systems (*24*). Phase I and phase II safety trials have successfully been completed with women and men in six countries (*25*-*27*), and a phase III effectiveness trial in South Africa (in collaboration with the University of Cape Town and the Medical University of Southern Africa) and Botswana (with the U.S. Centers for Disease Control and Prevention) will be initiated in late 2002.

Emmelle consists of the sulfated polysaccharide dextrin sulfate. It was developed by ML Laboratories (London, U.K.), and is currently being tested in phase I and II clinical trials by the Medical Research Council (London, U.K.) and the Institute for Tropical Medicine (Antwerp, Belgium) (*28*). Ushercell (consisting of cellulose sulfate) was developed by Polydex Pharmaceuticals (Scarborough, ON, Canada) and the Program for the Topical Prevention of Conception and Disease (TOPCAD, Chicago, Illinois, USA) (*29*). The latter is developing five other sulfated/sulfonated polymers. Several phase I clinical trials of Ushercell are currently being conducted by the Global Microbicide Project (GMP, Arlington, Virginia, USA), the HIV Prevention Trials Network (HPTN, Arlington, Virginia, USA), and the World Health Organization (*13*). Pro-2000 is being developed by

researchers at Interneuron Pharmaceuticals (Lexington, Massachusetts, USA); it is being tested in phase I and II clinical trials by the HPTN and the Medical Research Council (*30*). Pro-2000 will also be included in an upcoming multi-product phase II/III clinical trial in six different countries to be conducted by the HPTN (*13*).

3. Inhibitors of postfusion activity

A third class of compounds interrupts the HIV life cycle after the virus has infected its target cell (Table 3). They include compounds that inhibit HIV-specific enzymes that are required for viral replication in host cells, such as reverse transcriptase (RT) and protease. Others inhibit postbinding fusion.

Table 3. Inhibitors of post-fusion activity.

Product Category	Examples
Nucleoside/tide RT inhibitors	Tenofovir (PMPA), zidovudine (AZT)
Non-nucleoside/tide RT inhibitors	Carboxanilides (UC781), nevirapine
Protease inhibitors	Doxovir (CTC-96)
Postbinding fusion inhibitors	Lamivudine (3TC), didanosine (ddI), loviride

Tenofovir (Viread) was developed by Gilead Sciences (Foster City, California, USA) and was recently approved by the U.S. Food and Drug Administration for treatment of HIV infection (*31*). It is a nucleotide RT inhibitor that prevents HIV from entering the nucleus of healthy T cells. Gilead Sciences also formulated tenofovir as a gel for topical application, and it was shown to prevent SIV infection in macaques. Tenofovir is currently being tested in phase I and II clinical trials by the HPTN (*13*).

An example of a non-nucleoside RT inhibitor is UC781 (*32*). Extensive preclinical studies with UC781 have been completed, and Biosyn recently licensed the rights to develop it as a topical microbicide. Doxivir was originally developed as a treatment for herpes simplex virus and is currently being developed as a topical microbicide by Redox Pharmaceutical Corp. (Greenvale, New York, USA) (*33*). UC781 and the Doxivir-based microbicide have not yet reached clinical testing.

4. Compounds or products that enhance vaginal defense mechanisms

The normal vaginal flora of healthy women of childbearing age is dominated by lactobacilli. Lactobacilli produce a number of compounds that inhibit pathogenic microorganisms, including lactic acid, hydrogen peroxide, lactacin, and acidolin (*34*). These compounds also maintain a low, acidic pH in the vagina. Another important defense mechanism is naturally occurring antimicrobial peptides and antibodies in the vagina. A few newly developed microbicides aim to enhance these natural defense mechanisms (Table 4). Such products may be of particular relevance to those countries in sub-Saharan Africa where almost half the women of childbearing age have bacterial vaginosis, characterized by a lack of vaginal lactobacilli and an increased vaginal pH (*35*).

Table 4. Inhibitors of post-fusion activity.

Product Category	Examples
Maintaining normal vaginal flora	*Lactobacillus crispatus* suppository
Maintaining acid vaginal pH	BufferGel, Acidform gel
Antimicrobial peptides	Protegrins (e.g., IB-367), defensins, Gramicidin, peptidyl MIMs^{TM} , magainins
Monoclonal antibodies	Plantibodies, other biogenetically engineered monoclonal antibodies (e.g. Mab S19)

Researchers at Magee-Women's Hospital (Pittsburgh, Pennsylvania, USA) and GyneLogix (Louisville, Colorado, USA) have developed a suppository containing *Lactobacillus crispatus* to maintain or restore a healthy vaginal ecology. A phase I/II study in Pittsburgh found that regular insertion of the suppository allowed the hydrogen peroxide-producing lactobacilli to become part of the vaginal flora. The Medicines Co. (Cambridge, Massachusetts, USA) recently acquired the rights to further develop the suppository, initially for treatment of bacterial vaginosis (*36*).

BufferGel was developed by ReProtect (Baltimore, Maryland, USA). It is formulated to maintain the natural protective acidity of the vagina (pH < 5) by acidifying semen, which otherwise alkalinizes the vagina during and immediately after sex. The pH required for HIV inactivation has been reported to be between 4 and 5.8 in different studies, and many other STI pathogens are inactivated at a pH < 5 (*37*). BufferGel was found to be safe in phase I trials in five countries, and restored the normal vaginal flora in women with bacterial vaginosis (*38*, *38*). It will be tested in the upcoming HPTN multiproduct phase II/III effectiveness trial in six countries (*13*). Acidform gel (developed by TOPCAD and CEMICAMP, São Paulo, Brazil) is a bioadhesive gel with a similar mechanism of action and is in early clinical testing (*39*).

Gramicidin is a linear polypeptide antibiotic derived from *Bacillus brevis* soil bacteria. It was found to have potent anti-HIV activity in vitro (*40*). Gramicidin and other antimicrobial peptides could potentially enhance the local immune system in the vagina. They are undergoing preclinical and early clinical testing. Epicyte Pharmaceuticals (San Diego, California, USA) developed anti-HIV monoclonal antibodies, or plantibodies, that are genetically engineered from plants. They can be produced in large quantities at low cost and can be applied topically to offer passive local immunity against infection (*41*).

Complementary social science and market research on microbicides

Laboratory and clinical research on microbicides has been complemented by a growing body of social science and market research. Such research is essential to provide microbicide developers with critical information concerning how and whether a given product is likely to be used once approved. Social science research can be conducted over the course of the development process: prior to clinical trials, parallel to clinical trials, in conjunction with clinical trials, and after clinical trials have been completed. Social science research that has been conducted prior to or parallel to clinical trials includes research on attitudes of women and men to the concept of microbicides; hypothetical user preferences (after an explanation or demonstration of potential microbicidal products); and user preferences based on actual use of existing over-the-counter spermicides in different formulations (reviewed in *42*). Social science

research that has been conducted in conjunction with clinical trials includes research on acceptability of newly developed products being tested in trials; product use dynamics during trials; attitudes and experiences of men whose partners are participating in trials; and how best to educate trial participants on microbicides and other aspects of microbicide trials.

Most social science research to date has confirmed the urgent need women feel for an HIV prevention method that they can control, particularly in countries with a severe generalized HIV epidemic. In general, women and men in a variety of countries have expressed positive attitudes toward both contraceptive and noncontraceptive microbicides. Formulation preferences seem to differ by culture and context, and researchers should anticipate that multiple products would ultimately be required to meet the needs of all potential users (*42*).

Social science research that has been carried out independently of clinical trials includes research on harm reduction and dual protection strategies, and potential markets. The idea behind harm reduction is that risk may be reduced, even in situations when complete protection is not possible. One harm-reduction approach related to microbicides is hierarchical method counseling. In this approach, women are told that male condoms offer the best protection against HIV and other STIs, with the female condom as the next best option, followed by a diaphragm or cervical cap with spermicide, or spermicides alone. Research in this area has shown that offering multiple methods for HIV prevention generally increases the percentage of sex acts that are protected by at least one method and that successful condom users may supplement, but generally do not replace, condom use (reviewed in *42*).

When this research was carried out, it was assumed (based on in vitro and animal data) that currently available over-the-counter nonoxynol-9-containing spermicides are likely to have some protective effect against HIV, particularly when used in combination with a cervical barrier, even though phase III effectiveness trials of nonoxynol-9 had not yet been completed and cervical barrier devices had not yet been evaluated for their ability to block HIV infection. Since then, phase III effectiveness trials of nonoxynol-9 have shown that it can no longer be recommended for HIV/STI prevention. Therefore, only the first two options of the hierarchy remain until newly developed microbicides become available and cervical barrier devices have been evaluated for their ability to block HIV infection. It is not yet clear how microbicides will eventually fit into the hierarchy, because it will depend on their level of effectiveness against HIV and other STIs, and other factors that are not yet known.

Dual protection refers to protection from both HIV/STI infection and unwanted pregnancy. An important obstacle to dual protection is that those contraceptive methods with the best record of preventing pregnancy under typical conditions of use provide no protection against STIs. Condoms can effectively prevent both STIs and pregnancy if used consistently but are associated with relatively higher pregnancy rates than other contraceptives in typical usage situations. An alternative to using condoms alone is the use of two methods: condoms and an effective contraceptive method (for example, a hormonal method). It is not yet clear how newly developed microbicides will fit into the dual-protection mix, because it will depend on their level of effectiveness against HIV, other STIs, and unwanted pregnancy, and other factors that are not yet known.

To stimulate private-sector investment in microbicide research and development, a few microbicide market surveys were conducted by the public and nonprofit sector (see below). A market survey in the United States, conducted by the Alan Guttmacher Institute, estimated that 12.6 million American women would be "interested" in using a microbicide, and 7.7 million would be "very interested" (*43*). Another market survey

among urban women in 11 countries worldwide, commissioned by the European Commission, concluded that there is potentially a substantial commercial market for microbicides that is sufficient to justify further product development (*44*).

Conclusions and challenges

This summary of the microbicides pipeline and complementary research clearly shows that much progress has been made in the last decade. However, many challenges remain (*4*). First and foremost, a significant increase in investment from both the public and private sectors is needed to build on recent advances in the microbicides field. To date, no major pharmaceutical company has made a significant investment in microbicide research and development. Cited barriers for private-sector investment include scientific uncertainty about the concept, perceptions of a limited market and profit potential, competing demands for resources, regulatory uncertainty, and safety and liability concerns. In recent years, attitudes toward microbicides have generally become more positive in response to public and nonprofit initiatives to address these barriers. However, industry is not likely to make major investments in this area until proof of concept has been achieved. Public sector leadership therefore remains essential. Innovative public-private partnerships, similar to the International AIDS Vaccine Initiative (*9*), are also being explored.

Due to the lack of surrogate markers, phase III effectiveness trials of microbicides can only be conducted with large numbers of volunteers in populations with a high incidence of sexually transmitted HIV. Such populations can only be found in developing countries. As more products are moving into advanced clinical testing, streamlining of trials may be necessary to avoid overwhelming the limited number of available clinical trial sites and to reduce cost. Approaches that are being explored include trials with multiple product arms but only one or two control arms, and combined phase II/III trials instead of separate phase II and III trials.

Another major challenge is to ensure that microbicides, once proven effective, are available and accessible to all women who need them. Early planning is critical. For example, developers should aim for over-the-counter availability from the outset, and low-cost production methods should be considered early in the development process. International agencies and governments should begin to explore distribution networks, pricing mechanisms, local manufacturing, effective education strategies, streamlined regulatory processes, and increased awareness of and commitment to microbicides. A strategic multi-sectorial approach is critical to make microbicides available as rapidly as possible to the women who need them.

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